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13. SUPPLEMENTARY NOTES

14. ABSTRACT The hypothesis addressed by this project is that early life exposure to stress or glucocorticoids produces a distinct neurochemical and behavioral phenotype characterized by life-long vulnerability to stressors that trigger PTSD. Moreover, we hypothesize that the susceptibility to PTSD can be reversed in adult offspring by SSRI treatment. The goals for this year were to validate a model of prenatal stress, determine if the offspring exhibit behavioral and neurochemical phenotypes that are more responsive to stress and determine if exposure to traumatic stress (massed footshock; MFS) results in PTSD-like behaviors. We proposed to determine the effects of treatments with the SSRI sertraline in reversing the PTSD-like behaviors. We found a distinct behavioral and neurochemical phenotype in adult rats that had been exposed to prenatal stress. They exhibited greater response to stressful stimuli as measured by locomotor activity in a novel or stressful environment and had higher levels of dopamine and serotonin the neostriatum. However, MFS proved to be neither a valid nor useful model of PTSD. It failed to affect the most relevant behavioral measures, and confounded fear conditioning. Thus, we plan instead to test a modified Single Prolonged Stress (SPS) model that has been reported to elicit relevant behavioral effects and retains the temporal features of MFS that made it amenable to acute pharmacological intervention. We will then test sertraline in this model.

15. SUBJECT TERMS
Rats, prenatal stress, massed footshock, PTSD, open field test, social interaction test, fear conditioning, extinction, glucocorticoid receptors, dopamine, serotonin.

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A. INTRODUCTION:

Traumatic stress is a requirement for the development of PTSD. However, the majority of trauma-exposed persons do not develop PTSD. Therefore, examination of the typical effects of a stressor may not identify the critical components of PTSD risk or pathogenesis. Instead, PTSD represents a unique phenotype associated with failure to recover from the ordinary effects of trauma. One obvious explanation for individual differences in vulnerability to PTSD is that there may be genetic predisposition to susceptibility to precipitating stressors. However, to date, very few genetic polymorphisms for PTSD have been identified. An alternative mechanism that would impart lifelong vulnerability to PTSD is epigenetic alteration in gene expression programmed by exposure to early life stressors. Therefore, the hypothesis to be addressed by this project is that early life exposure to stress or glucocorticoids produces a distinct neurochemical and behavioral phenotype characterized by life-long vulnerability to stressors that trigger PTSD. Moreover, we hypothesize that the susceptibility to PTSD is programmed epigenetically by early life trauma and can be reversed in adult offspring by SSRI treatment. To address this hypothesis, we proposed the following specific aims: 1. To generate and characterize four animal models of early life stress: prenatal stress; perinatal stress; prenatal dexamethasone; and perinatal dexamethasone. 2. In each model, to determine adult predictors of vulnerability to stress: Adult offspring of the four models developed in Specific Aim 1 will be tested on behavioral, physiological, molecular and neurochemical measures. 3. To determine adult vulnerability to stress: Adult offspring of the four models developed in Specific Aim 1 will be exposed to traumatic stress (massed footshock). Behavioral, physiological and molecular neurochemical measures will be made. 4. To determine the effects of treatments with the SSRI, sertraline, in the four models developed in specific aim 1: Epigenetic programming of hippocampal glucocorticoid receptor expression is believed to be mediated through serotonergic mechanisms and can be reversed by SSRIs. Osmotic minipumps will be used to chronically deliver the SSRI sertraline to the four rat models and their respective controls. Behavioral, physiological and molecular/neurochemical measures of PTSD-like phenotypes will be made.

B. BODY:

During this initial funding period we performed experiments to establish the methods for two of the three tasks outlined in the statement of work: 1) Determine adult predictors of vulnerability to stress; 2) Determine adult vulnerability to stress.

Experimental Design. We assessed the prenatal stress model by immobilizing timed-pregnant female rats on embryonic days (ED) 14 – 21 for one hour. Unstressed pregnant females served as a control. On post-natal day (PD) 3, the litters were culled to 8 pups. Males were weaned and pair-housed on PD 21. On PD 42, half the animals were exposed to a mild foot shock stress. We made a minor modification to the foot shock procedure in which we substituted a shock probe in a cage with bedding so that we could measure the extent to which the animals buried the probe with cage bedding after receiving a mild shock. The extent of burying behavior is associated with greater reactivity to stress. Immobility (i.e., "freezing") in these rats is measured as well as this provides a separate measure of reactivity. We initially also exposed a group of animals to a single immobilization stress to measure the effect of this stress on their response to a battery of tests. We have not finished collecting data on this group. On day 54, we exposed the mice to a PTSD stress model which consists of mass foot shock administered 3 times. On PD 70, half the animals were sacrificed for a number of neurochemical measures. The other half is then assessed

for stress reactivity with a battery of tests on PD days 70-79. On day 73, animals are tested for hyperarousal by measuring locomotion in an open field. On day 74, the rat is exposed to another rat in the open field to measure social interaction. On day 75, state anxiety and stress reactivity are tested in the elevated plus maze. On day 76, fear conditioning is measured and on days 77 through 79, extinction of the conditioned fear is tested. Because of the logistics involved in testing a large number of animals on this battery of tests, we have performed the above with separate groups of animals and combined the data from the groups. Not all of the tests have been completed at this point, so we will present only the data for which we have enough samples for statistical analysis of the results.

Results.

Figure 1. shows the effect of prenatal stress on reactivity to foot shock in the shock probe defensive burying test.
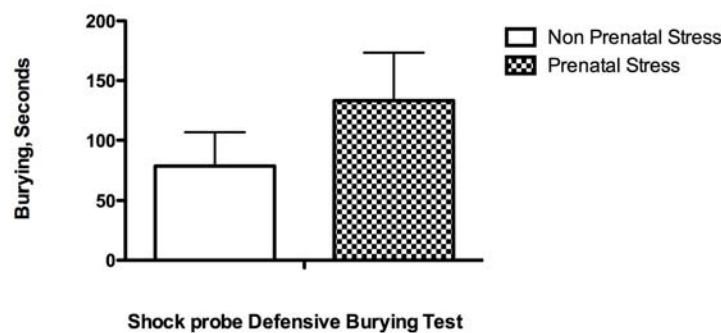

Figure 1. Effect of prenatal stress on the shock probe defensive burying test. The data represent the mean ± SEM for 7 – 10 animals.

Although it appears as though the offspring exposed to maternal prenatal stress show relatively greater burying activity, the difference between the two groups did not reach statistical significance.
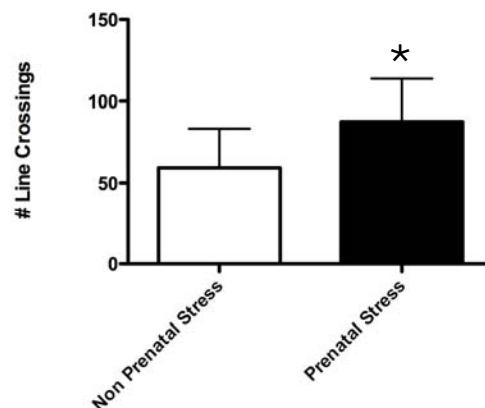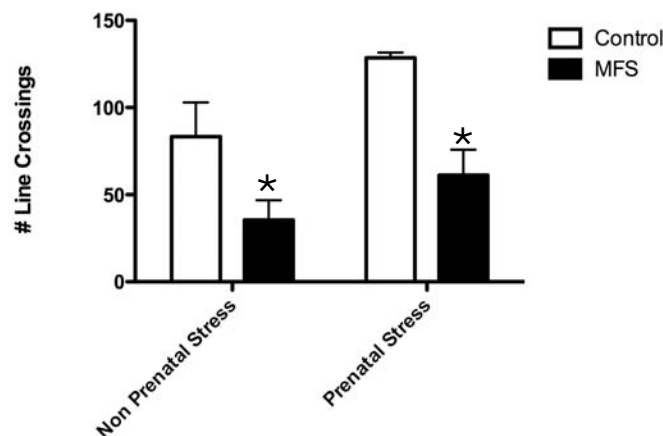

Figure 2. Effect of prenatal stress on adult locomotor activity. The data represent the mean ± SEM for 12-14 animals. *, $p < 0.05$, significantly different from non prenatal stress.

The effect of prenatal stress on locomotor activity in the open field is shown in Figure 2. Offspring exposed to maternal prenatal stress exhibit significantly greater locomotor activity in the open field. However, there was no effect of prenatal stress on the social interaction component of the test battery.
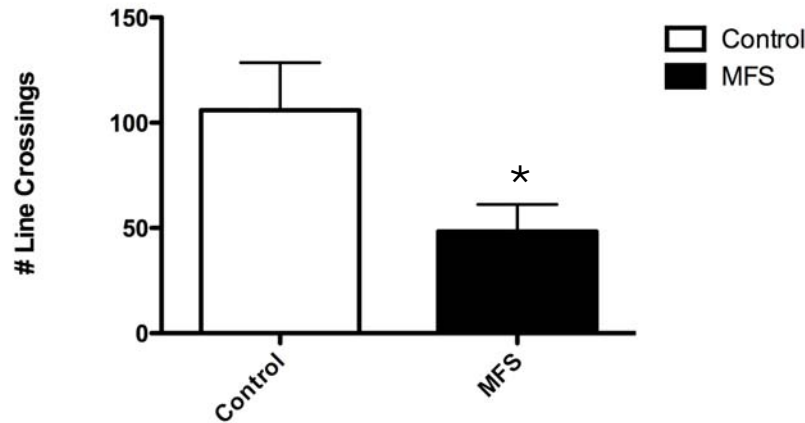
Figure 3. The effect of massed foot shock on locomotor activity in the open field. The data represent the mean ± SEM for 12-14 animals. *, $p < 0.05$, significantly different from control.
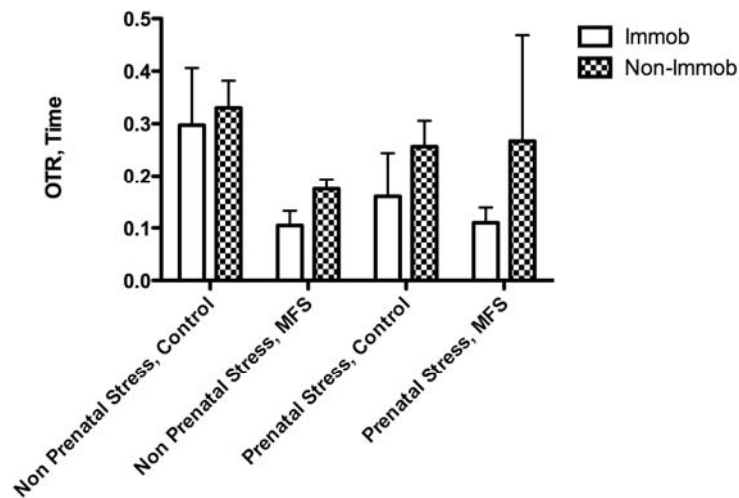
The effect of massed foot shock on activity in the open field is shown in Figure 3. The mean number of line crossings in the open field was significantly reduced in mice after massed foot shock. However, as shown in Figure 4, there was no significant interaction with prenatal stress.

Figure 4. The effect of massed foot shock on locomotor activity in the open field. The data represent the mean ± SEM for 12-14 animals. *, $p < 0.05$, significantly different from control.

Although massed foot shock was associated with a decrease in open field activity, and prenatal stress was associated with an increase in open field activity, there was no significant interaction. Thus, exposure to prenatal stress did not potentiate the effects of massed foot shock.
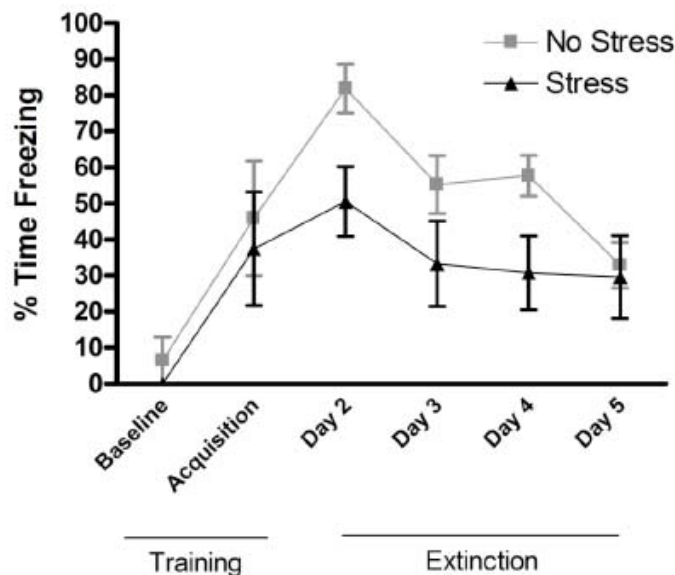
Figure 5 shows the effects of prenatal stress on the response to massed foot shock on the number of entries in the open arm of the elevated plus maze.

Figure 6. The effect of massed foot shock and prenatal stress on stress reactivity in the elevated plus maze. The data represent the mean ± SEM for 3 to 4 animals.

There was no significant interaction between prenatal stress and MFS on immobilization-induced anxiety in the elevated plus maze.

We had proposed to examine the effects of MFS on fear conditioning and extinction. However, we found a significant confounding effect of MFS on this test. Shown in Figure 7 are the effects of MFS on fear conditioning and extinction.

Figure 6. The effect of massed foot shock and prenatal stress on fear conditioning and extinction. The data represent the mean ± SEM of the percent of the time spent freezing in response to a tone that was paired with a shock.

As shown in Figure 6, MFS actually decreased the response to the fear conditioning task. We hypothesize that MFS accustomed the rats to shock. Thus, when they were exposed to the milder shock in the fear conditioning and extinction test, they reacted less to the aversive stimulus.

We also made neurochemical measurements. While they are still in progress, we have included the results so far.
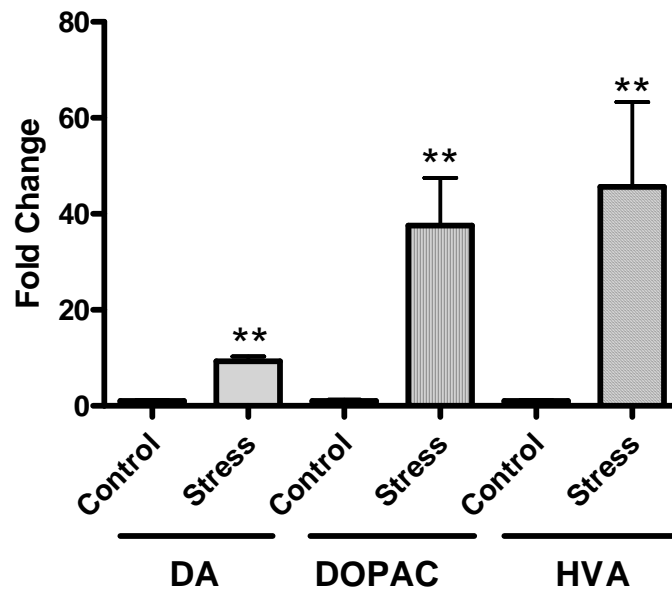
Figure 7: Effect of prenatal stress on neostriatal DA and metabolites. The data represent the mean ± SEM for 4-7 animals. *, p<0.05, significantly different from non prenatal stress.

Figure 7 shows that prenatal stress greatly increases dopamine and its metabolites in the striatum of adult rats that are the offspring of mothers who were subjected to immobilization stress during pregnancy. These data are consistent with the increased locomotor activity in these rats.
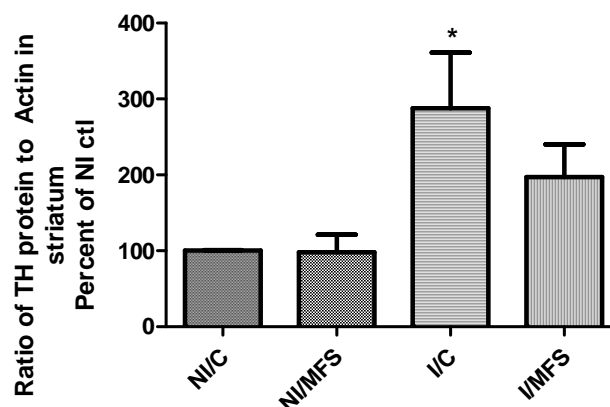
Figure 8: Effect of prenatal stress on neostriatal TH protein. The data represent the mean ± SEM for 4-7 animals. *, p<0.05, significantly different from rats that were not immobilized in utero.

We plan to repeat the measures in the striatum, but these data are consistent with the results of measures of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine, shown in Figure 8, showing a significant increase in neostriatal TH protein.
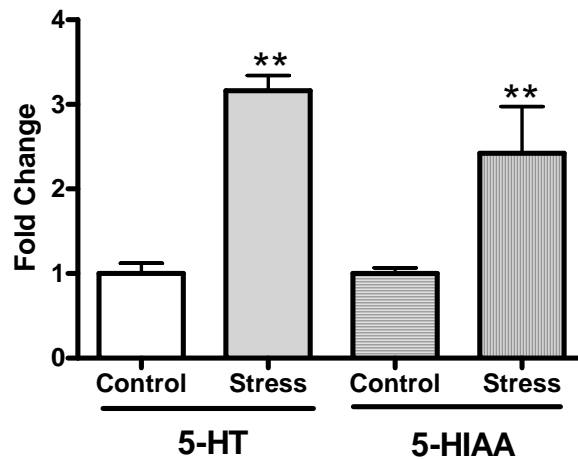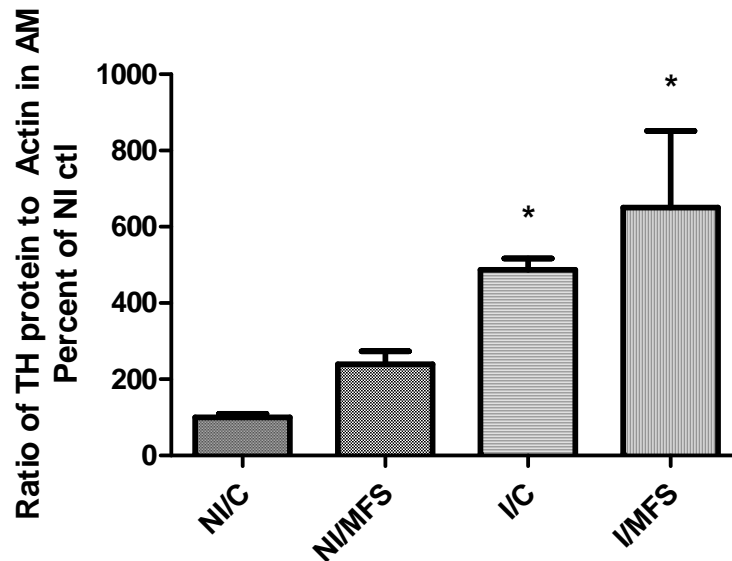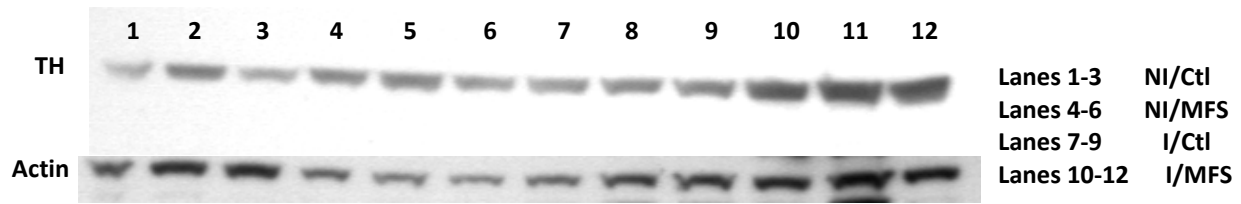

Figure 9: Effect of prenatal stress on neostriatal serotonin and its metabolite. The data represent the mean ± SEM for 4-7 animals. **, p<0.01, significantly different from non prenatal stress.

Furthermore, Figure 9 shows that serotonin and its metabolite were also significantly increased in the neostriatum.

We also measured tyrosine hydroxylase mRNA and protein in the adrenal medulla as a measure of peripheral sympathoadrenal activation (Figures 10 and 11).
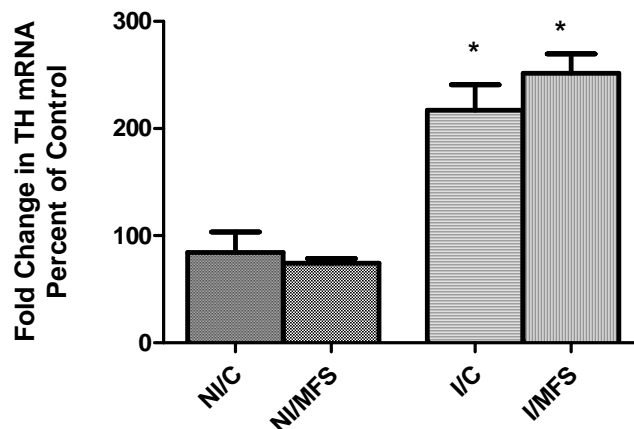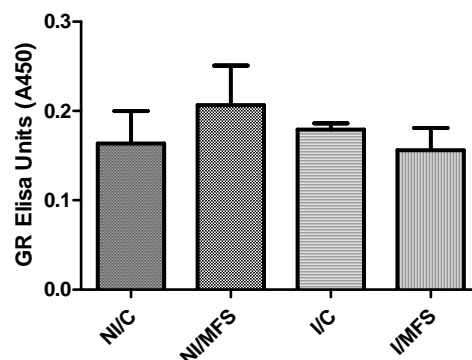

Figure 10: Effect of prenatal stress on adrenal TH mRNA. The data represent the mean ± SEM for 3-4 animals per group . **, p<0.01, significantly different from non prenatal stress.

1	2	3	4	5	6	7	8	9	10	11	12

TH

Actin

Lanes 1-3 NI/Ctl
Lanes 4-6 NI/MFS
Lanes 7-9 I/Ctl
Lanes 10-12 I/MFS

Figure 11: Effect of prenatal stress on adrenal TH protein. The data represent the mean ± SEM for 3 animals per group . *, p<0.01, significantly different from non prenatal stress.

The results show that prenatal stress increased both TH mRNA and TH protein in adult offspring. This is consistent with a behavioral phenotype that would be hyper-responsive to stress and is consistent with the behavioral data showing that prenatal stress increases behavioral responsiveness to mild stressors, e.g. the defensive burying shock probe test.

Figure 12: Effect of prenatal stress on glucocorticoid receptors in the hippocampus. The data represent the mean ± SEM for 3-4 animals per group . *, p<0.01, significantly different from non prenatal stress.

It has been reported that stress early in life reduces the number of glucocorticoid receptors in the hippocampus in adult. The increase in these receptors causes elevation of glucocorticoids in response to stress, because of reduced feedback inhibition of ACTH production. Therefore, we also measured glucocorticoid receptors in the hippocampus,. The results are shown in Figure 12. There were no differences in hippocampal glucocorticoid receptors as measured by an ELISA assay for glucocorticoid receptor protein.

KEY RESEARCH ACCOMPLISHMENTS:

- **We established a reliable protocol for inducing prenatal stress**
- **We developed a thorough, valid, consistent and informative test battery for measuring adult predictors of stress.**
- **The prenatal stress model produces a phenotype of stress reactivity in adult offspring and a predisposition to certain aspects of PTSD, including increased reactivity to the open field component of the social interaction test and increased reactivity to mild foot shock. The prenatal stress model also produces a distinctive neurochemical phenotype characterized by elevated neostriatal dopamine, serotonin and metabolites, increased tyrosine hydroxylase (TH) expression in the neostriatum and increased TH mRNA and TH protein in the adrenal medulla. These neurochemical phenotypes are consistent with the increased stress reactivity and elevated locomotor activity in the open field.**
- **We have established that massed foot shock model interferes with testing of fear conditioning and extinction, which is a key feature of PTSD that we would like to model – Therefore, we plan to test other models of traumatic stress e.g., the chronic unpredictable stress model.**

REPORTABLE OUTCOMES:

An abstract of a preliminary report of this work was presented in Kansas City on September 2, 2009.

Randy Strong, David Morilak, and Alan Frazer (2009) Mechanisms of vulnerability to PTSD: the role of early life stressors. Military Health Research Forum, Kansas City MO.

CONCLUSION: We have established a model of prenatal stress that produces an adult phenotype characterized by changes in neurochemistry in the peripheral and central nervous system and increased stress reactivity in the open field. Moreover, it appears to increase reactivity in the shock probe defensive burying test. The latter will require adding additional animals to reach statistical significance, but the results so far are consistent with increased reactivity of rats exposed to prenatal stress to mildly stressful stimuli. However, our PTSD-like traumatic stress appears to be deficient in several ways. It doesn't show an interaction with prenatal stress in the open field or in the immobilization-induced effects on exploratory activity in the elevated plus maze. It also appears to have introduced a confounding variable in the fear conditioning and extinction test. Thus, in the future we plan to try an alternative PTSD-like stress, such as chronic unpredictable stress.

REFERENCES:

None

APPENDICES:

None

SUPPORTING DATA:

Shown in the body of the report.